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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR. — No. 179.

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WITH A PLATE.

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In 1903 F. Meves published a short account of spermatogenesis in the honey bee in which it was held that the process was remarkably unlike that of other animals, and simulated in an interesting way the maturation of the female sexual products.

As is well known, an important parallelism between the formation of polar cells in the maturing egg and the last two cell divisions leading to the formation of spermatozoa was established for Ascaris by O. Hertwig, and has since been shown to be a general condition of maturation throughout the animal kingdom. But while in the sexual cells of the female maturation results in the formation of one functional and three (or two) non-functional elements, in the male the usual outcome is four elements, the spermatozoa, all of which are functional.

In the honey bee Meves showed that the maturation divisions of the primary spermatocytes resulted, as in the case of the primary ovocyte generally, in the production of a single functional cell, inasmuch as there are produced from the primary spermatocyte in succession two "Richtungskörper." However, this fundamental difference was noted: whereas in the formation of polar cells during the maturation of the primary ovocyte there are produced two nucleated "Richtungskörper," in the spermatogenesis of the honey bee only the second of the corresponding bodies is nucleated, the first one being composed exclusively of cytoplasm.

At the time Meves published these observations we had already begun the study of the germinal cells in the male honey bee, and having now arrived at somewhat different conclusions from those set forth by him, will give here a preliminary account of our results thus far, the intention being to publish later a more detailed and comprehensive paper on the subject.

In the final generation of spermatogonia in the honey bee there is at the apex of each conical cell a spheroidal, nearly homogeneous body, which represents the remnants of the interzonal filaments of the preceding cell division. These bodies are stained black in iron haematoxylin, and on being washed out assume a characteristic yellowish gray color. Since they are admittedly the metamorphosed remnants of filamentous structures first named by Mark ('**81**, pp. 198, 539) interzonal filaments, we shall henceforth speak of them as the *interzonal bodies*. The interzonal body is identical with the "Zellkoppel" of Paulmier ('**99**, p. 228), to which Prowazek (:**01**, p. 201) has given the name "Spindelrestkörper." It is to be noted that the term "Zellkoppel" was used by Zimmermann ('**91**, p. 189), who introduced the name into cytology, with a somewhat different meaning from that employed by Paulmier. This is in itself a reason for applying another name — interzonal body — to this structure.

At the end of the growth period, which follows the last spermatogonial division, the cells have increased greatly in size and have become in general spherical, a form which is more or less modified by the mutual pressure of the closely packed elements. At this stage (Figure 1) the interzonal body (x) is clearly visible, and is in contact with the cell membrane. Meves shows it in his Figure 1, but makes mention of it neither in his explanation of the figure nor in the accompanying text.

The first evidence of spermatocyte division is seen when the centrosome, which lies in contact with the cell membrane, divides and the two daughter centrosomes move apart along the periphery of the cell. The centrosomes during their migration appear to exert a marked influence on the form of the cell, which exhibits two more or less conspicuous elevations, the apex in each case being occupied by one of the centrosomes. Figure 2 represents a fairly early stage in the migration of the centrosomes and shows clearly the marked change in the form of the cell due to their presence. The distance between the two centrosomes increases until these ultimately arrive at opposite poles of the cell (Figure 3). Up to this time each centrosome seems to have exercised nearly the same amount of influence as the other in modifying the form of the cell; but from this time forward the influence of one is seen to predominate over that of the other, until at length (Figure 4) one end of the cell is drawn out into a long, slender, slightly tapering, finger-like process, at the tip of which is located the centrosome. This centrosome will be designated as the proximal one, the other as the distal centrosome.

The choice of these designations rests on a later condition in the

arrangement of the spermatocytes. These become grouped in the cyst in a radial manner with their interzonal bodies clustered at the centre, very much as the spermatogonia are arranged in their follicles. This constant orientation of the cells in relation to the cluster as a whole makes it possible to use without ambiguity the terms proximal and distal.

The nucleus, which hitherto has been near the centre of the spermatocyte, now changes its position, migrating to the distal end of the cell; it generally comes to lie close to the corresponding centrosome. Coarse fibres, which stain deeply in iron haematoxylin, now make their appearance, extending from the distal centrosome around the nucleus to the region of the proximal centrosome. At this time the finger-like process terminating in the proximal centrosome may be reduced to a very narrow cytoplasmic stalk (Figure 4), so that it is difficult to observe whether the fibres at this stage actually reach the centrosome or not. It is, however, probable that they do, as they may be traced for some distance into the base of the finger-like projection.

Changes, which we shall not attempt to describe here in detail, have meantime taken place within the nucleus. The chromatin passes through a spireme stage and gives rise — apparently by segmentation and a concentration of the segments — to sixteen double, dumb-bell shaped chromosomes (Figure 5), which lie in rather scattered positions. The bivalent structure of the chromosomes becomes less conspicuous when, a little later, the nucleus enters on the metaphase.

At about this stage the nucleus elongates, first in the direction of the distal end of the cell (Figure 7), and later at the opposite end, until finally it becomes spindle shaped, extending more or less completely from pole to pole (Figure 8). At the time of nuclear elongation a few thick, deeply staining intranuclear-spindle fibres are seen connecting the chromosomes with the distal centrosome, and later, as the nucleus increases in length, similar fibres also run to the proximal pole of the cell. These fibres are clearly separate from one another in the region of the chromosomes, but as they approach the centrosomes they seem to coalesce, until it is impossible to make out separate elements. In the cell represented in Figure 8, a bundle of fibres is traceable from the chromosomes to the distal centrosome, and on the opposite side of the chromosomes a bundle may be followed for some distance into the base of the finger-like process which terminates in the proximal centrosome. In other cases the nucleus, although much attenuated at the poles, has been clearly seen to stretch from one centrosome to the other, the chromosomes being midway between the two centrosomes. I find nothing to corroborate the condition

shown by Meves in his Figure 2, where the elongated nucleus with its contained spindle figure is entirely isolated from the two centrosomes of the cell. Apparently he has overlooked the fibrous prolongations which connect the ends of the spindle with the centrosomes. The coarse, granular, extranuclear fibres already referred to are still to be seen occupying the greater part of the cell, extending in somewhat irregular sinuous courses outside the nucleus from pole to pole.

The mitotic figure here described is evidently that of the first spermatocyte division; but now a peculiar phenomenon occurs. Instead of a division of the chromosomes taking place, as would be expected, the figure does not for the present progress beyond the beginning of the metaphase. The spindle fibres either break down, or, what is more likely, become closely pressed together into a single bundle; but the chromosomes maintain their form for some time. A cross section through the middle of the spindle at this stage (Figure 6) shows the chromosomes still surrounded by a nuclear membrane, and likewise the cut ends of the cytoplasmic fibres lying outside that membrane.

The interzonal body persists throughout the period of the nuclear changes last described without any apparent alteration in form or size, but toward the end of this period it has a position at, or very close to, the proximal end of the cell, and soon afterward it is found directly beneath the proximal centrosome. Eventually it occupies the base of the finger-like process of cytoplasm (Figure 9) previously described. There is some variation in the sequence of phenomena during this period. As early as the stage shown in Figure 7, the centrosome and the interzonal body are sometimes seen closely applied to each other, while in other cases at a much later stage in the nuclear metamorphosis (Figure 9) a portion of the finger-like process may still be seen between the two. At length the interzonal body occupies the place of the finger-like process of cytoplasm and protrudes from the main body of the cell (Figure 10), the centrosome continuing to occupy its former position at the proximal end of the projection. At this stage the interzonal body is a spherical object supported on a cylindrical process of the cell, and resembles superficially the first polar cell formed during the maturation of eggs; but it differs from the polar cell in obvious particulars. Not only is it destitute of visible chromatic substance and the usual remnants of interzonal filaments, but it exhibits from the beginning a fairly well-defined outline on the side toward the body of the cell. Except for the persisting centrosome, its substance is homogeneous and slightly more refractive than the neighboring cytoplasm.

During the migration of the interzonal body into the finger-like cell process, other portions of the cell undergo conspicuous changes. The extranuclear fibres which, in the earlier stages, extended from the distal centrosome around the nucleus and the interzonal body to the proximal centrosome, have now increased in number, and crowd upon the nucleus. The latter continues to change in form and size; it becomes considerably flattened and much smaller. The chromosomes are crowded together so that they often appear (Figure 10) as a single deeply staining mass, generally much nearer the proximal than the distal end of the cell, and sometimes actually in the projection of the cell close to the interzonal body. Careful decolorizing at this stage reveals the chromosomes in the form of dyads, with possibly an actual separation of the dyads into their components. This perhaps represents what Meves describes as the breaking up of the chromosomes into granules.

Soon after this the cylindrical process of cytoplasm connecting the interzonal body with the main portion of the cell becomes elongated and more attenuated. Eventually it assumes a slender neck-like form, and then by rupturing, effects a complete detachment of the interzonal body from the spermatocyte (Figure 11). The region of greatest attenuation and final rupture is such that a small, though possibly variable, amount of undifferentiated cytoplasm is cut off with the interzonal body. The rôle of this body in spermatogenesis is now finished; apparently it gradually diminishes in size (Figure 15), and ultimately disappears entirely.

The extranuclear fibres, which were well developed as far back as the stage represented in Figure 4, are possibly concerned in some way with the elimination of the interzonal body; they are at no time connected with any chromatin, and yet they are as large and stain as deeply as the fibres within the nucleus. They persist and are to be recognized in the cell throughout the succeeding division.

At about the time of the detachment of the interzonal body the nuclear membrane disappears and the spindle presents a form resembling that which usually characterizes such a structure. The chromosomes are now seen to be in the metaphase (Figure 11), having returned to the condition which they exhibited immediately before the elimination of the interzonal body.

The history of the nucleus during the elimination of the interzonal body is hard to trace and difficult to interpret. Meves states that, after the formation of a spindle figure inside the persisting nuclear membrane, with sixteen chromosomes irregularly arranged at the equator, there is no further progress; that, on the contrary, the chro-

mosomes remain clustered together in one place, and finally break up into granules; and that the spindle fibres are converted into a network. While we agree with the main point implied in this description, — viz., that the chromosomes do not separate into two groups representative of two daughter nuclei, — our observations lead us to think that the nuclear matter does not pass through a true reticular stage. For the chromosomes retain more or less distinctly their individuality, and the spindle fibres do not seem to take on the reticulate condition, but rather, to become confluent into a smaller number of strands. Whatever may be the method of its formation, however, the spindle and its chromosomes present almost exactly the same conditions immediately after the elimination of the interzonal body that they did just before that event. We have found, then, in our material no evidence of a typical prophase leading up to the formation of the spindle which is connected with the production of the second (the nucleated) bud.

During the period intervening between the formation of the first and second buds the centrosomes sometimes present a condition that may have a bearing on the interpretation of the process we are dealing with. Either the distal or the proximal centrosome, or rarely both, becomes elongated in a plane perpendicular to the axis of the spindle (Figures 10, 11), and in some instances a constriction divides it more or less completely into two bodies.

When the interzonal body has been detached, the spindle figure again assumes the condition characteristic of a nucleus in the metaphase; its cross section becomes greater, and the chromosomes, sixteen in number, immediately undergo division. The small daughter chromosomes, of the same size as each of the components of a dyad previously described, migrate to the poles of the spindle, leaving stretched between them interzonal filaments, which stain in iron haematoxylin like the spindle fibres. Nearly up to the stage represented by Figure 12, nothing suggests an unequal division of the cell; but as soon as the chromosomes arrive at the poles of the spindle the proximal end of the cell elongates and the chromosomes appear as a deeply stained mass at the apex of a second finger-like process of cytoplasm. The chromosomes at the opposite pole become surrounded by a membrane, thus giving rise to a small typical nucleus. Between these two nuclear bodies are seen the interzonal filaments together with remnants of the more peripheral fibres already described in connection with the account of the elimination of the interzonal body. The finger-like process at the proximal end of the cell with the nuclear mass at its tip continues to elongate (Figure 14), and eventually its

terminal part, containing the chromatin, is detached in the form of a very small cell, — much smaller, indeed, than the interzonal body previously set free. We have called this a cell, though it is difficult to determine how much, if any, undifferentiated cytoplasm is cut off with the nuclear matter. This, however, is a difficulty which is often encountered in studying the polar cells formed during the maturation of eggs, and does not in our opinion preclude the use of this designation. When fully separated from its sister cell, this small cell assumes a spherical form and increases in size. The nucleus of the larger cell, the spermatid, likewise increases in size and becomes to all appearances the counterpart of the detached polar cell. Figure 15 represents the two cells at this stage, and it also shows the previously eliminated interzonal body, now much diminished in size. The chromatin in both nuclear bodies becomes variously aggregated, but assumes in general a peripheral position; the metamorphosis of the larger cell — the spermatid — now begins.

Meves in his description of the first "Richtungskörper" is evidently dealing with what we have called the interzonal body, the peculiar history of which has been briefly described. If this so-called bud were in truth made up simply of undifferentiated cytoplasm, as Meves leads us to believe, perhaps one would be justified in considering it a rudimentary spermatocyte of the second order, even though it contained no chromatin. But if it is composed of the remnants of the interzonal filaments, which have become metamorphosed into a definite body within the cell, and is surrounded by only the slightest amount of undifferentiated cytoplasm, if any, it is difficult to find any sufficient basis for homologizing it with the first spermatocyte division of typical spermatogenesis. Moreover, such a casting forth of spermatogonial spindle remnants has already been observed in cases where four functional spermatids are formed in the normal way, and where, consequently, this elimination of interzonal matter can have nothing to do with the first spermatocyte division. Paulmier ('**98**, p. 228) has described such conditions in Anasa tristis in the following terms: "At the tip of the resting cell [spermatogonium] is a structureless mass which is formed from the remains of the intermediate spindle fibres [interzonal filaments] of the preceding division." Further on he adds, "As a group of chromosomes approaches the end of its movement the cell loses its conical shape and becomes more cylindrical, and the Zell-Koppel [interzonal body] of the preceding generation is cast off, remaining for a time as a small isolated mass, which ultimately disappears." Blackman (:**05**, p. 37) likewise describes a somewhat

similar phenomenon in the male germ cells of Scolopendra, where the spindle remnants of both the first and the second spermatocyte divisions are eliminated during a rotation of one daughter cell upon the other.

It seems possible, therefore, that this globule, composed principally of the substance of the interzonal filaments, is not comparable with the spermatocyte of ordinary spermatogenesis, and therefore not with a polar body in ovogenesis.

The smaller of the two bodies resulting from the real mitosis which follows the elimination of the interzonal body bears a striking resemblance, it must be admitted, to the polar cell of eggs, and it is unquestionably the result of a true, though modified, cell division. This phenomenon seems, however, never to have been seen in the spermatogenesis of any other animal. There is here an equal division of chromatin accompanied by a very unequal division of the cytoplasm, precisely as in the formation of the polar cell in eggs. The small cell apparently begins to undergo a metamorphosis parallel to that of its larger sister cell, as Meves has maintained. Although Meves believes that the small cell eventually degenerates, positive evidence of this has not yet been produced. If it is to be interpreted as the homologue of a polar cell, the question at once arises, Which of the two polar cells usually produced does it represent? From the standpoint of Meves, the first body might be looked upon as a partially abortive attempt to produce the equivalent of the first polar cell, and the second body might then be regarded as in some sense the equivalent of the second polar cell; but even were his view about the first (interzonal) body correct, the division of nuclear substance accomplished by the formation of the second bud would more strongly point to this, rather than the first, as being the homologue of the first polar cell. That view is, perhaps, strengthened by the facts here presented, which tend to show that the body first produced has nothing whatever to do with cell division, and that in the occasional doubling of the centrosome (Figures 10, 11) some progress is made toward a second cell division, which, if completed, would result in the formation of either two spermatids or a second true polar cell. However, it must be admitted that, on the assumption that the interzonal body has nothing to do with an attempted cell division, the peculiar changes passed through by the chromatin preceding and during the period of the elimination of that body remain unexplained and without a parallel.

On the other hand, the beginning metamorphosis of the small nucleated body (second bud) suggests that it is the equivalent of a spermatid rather than a spermatocyte of the first order, and this view

is strongly supported by the statement of Meves that in Vespa germanica the cell division corresponding to this results in the production of two spermatids of equal size.

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EXPLANATION OF PLATE.

All figures were drawn with the aid of a camera lucida and are magnified 2073 diameters.

FIGURE 1. Primary spermatocyte, resting stage.

FIGURE 2. Primary spermatocyte; the two centrosomes moving apart.

FIGURE 3. Primary spermatocyte; the centrosomes have arrived at opposite poles of cell.

FIGURE 4. Proximal finger-like process of the cell, terminating in the proximal centrosome; from the distal centrosome extend prominent extranuclear fibres.

FIGURE 5. Nucleus of primary spermatocyte, showing sixteen dyads.

FIGURE 6. Cross section of a primary spermatocyte in the same stage as that of Figure 9.

FIGURE 7. Elongation of the nucleus toward the distal centrosome.

FIGURE 8. The nucleus exhibits a spindle-shaped elongation, and intranuclear spindle fibres are established.

FIGURE 9. The interzonal body has migrated to the base of the finger-like process.

FIGURE 10. The interzonal body in the course of being constricted off from the cell.

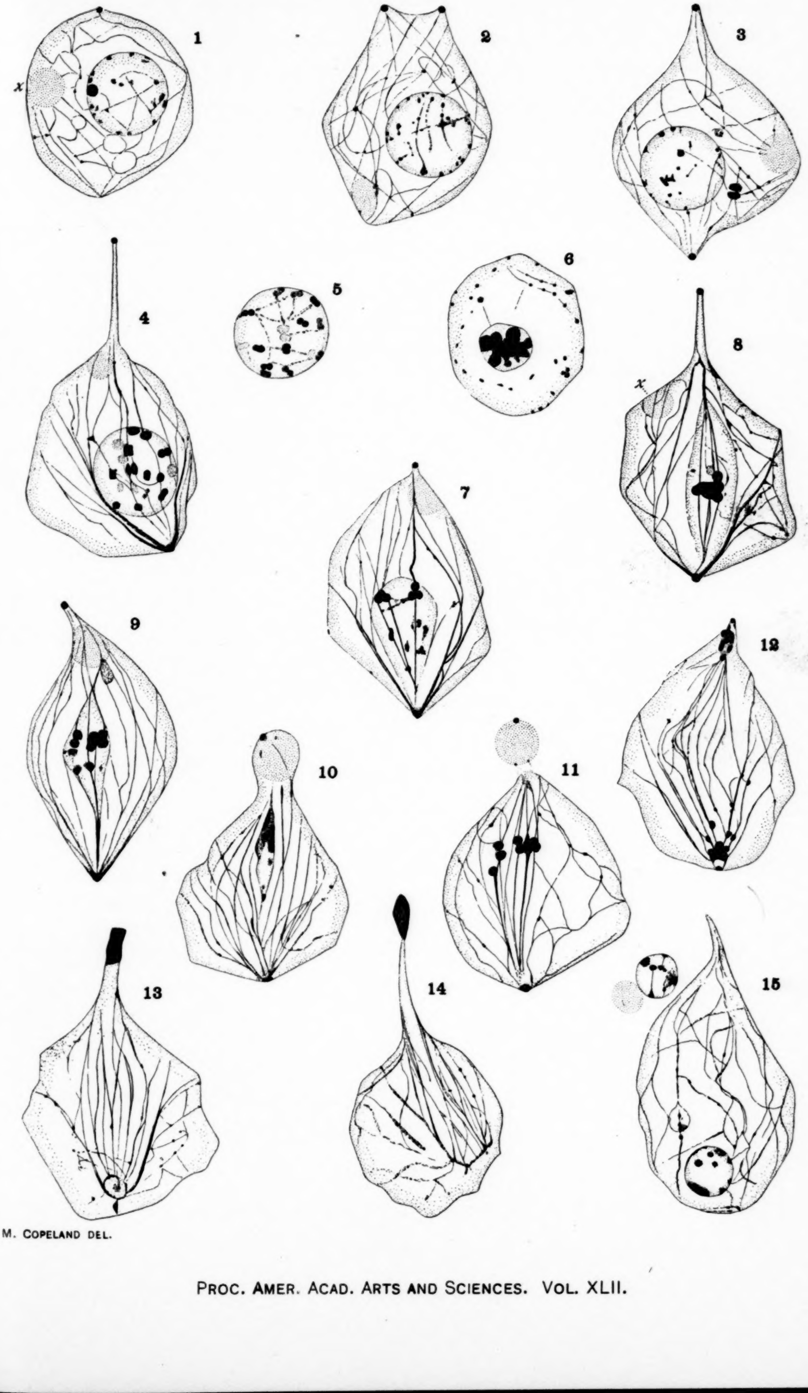
FIGURE 11. The interzonal body is detached, and there is a typical spindle in the beginning of the metaphase.

FIGURE 12. Late anaphase.

FIGURE 13. Finger-like process surmounted by chromatin of polar cell.

FIGURE 14. Chromatin of finger-like process nearly detached from the process.

FIGURE 15. Spermatid, interzonal body reduced in size, and polar cell.



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